these measurements to a series of T-domain mutants with substitutions in His residues involved in modulating pH-dependent refolding and insertion of the protein. The comparison of the results with those obtained in cell cytotoxicity assay performed with full length toxin revealed lack of correlation of the leakage-based assay with other assays. Our data suggest that release of fluorescent markers is related to the stress induced by the interfacial binding, rather than by the insertion of the protein in a functional conformation. To resolve the contradiction between cellular and in vitro measurements we have developed a translocation test based on the cleavage of the N-terminal part of T-domain upon its translocation into thrombin-loaded vesicles. Application of this test to a series of mutants correlated well with results of cytotoxicity. In the other set of experiments we have investigated lipid dependence - nonlinear changes with surface potential (which is in contrast with leakage measurements) confirming our previous suggestion that formation of the final inserted state is modulated by anionic lipids. NIH GM069783 (ASL), GM-29210 (AF), AI-022021(RGC) and Fulbright Foundation.

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Replacement of C-Terminal Histidines Uncouples Membrane Insertion and Translocation in Diphtheria Toxin T-Domain

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The translocation (T) domain plays a key role in the action of diphtheria toxin and is responsible for transferring the N terminus-attached catalytic domain across the endosomal membrane into the cytosol in response to acidification. The T-domain undergoes a series of pH-triggered conformational changes which take place in solution and on the membrane interface, and ultimately result in transbilayer insertion and N terminus translocation. Structure-function studies along this pathway have been hindered because the protein population occupies multiple conformations at the same time. Here, we report that C-terminal histidine residues H322, H323 and H372 of the isolated T-domain are important for the effective transition from the inserted intermediate to the functional open-channel state in the insertion/translocation pathway. We have mutated these histidine residues into triple-Q, double-Q and single-Q mutants and followed their behavior along the insertion/translocation pathway by fluorescence and CD spectroscopy and functional assays in membranes. Triple, double and single mutations caused a loss of characteristic conductance in planar bilayers in different degrees, as well as perturbation in the fold of the inserted state of T-domain relative to the WT T-domain. Interestingly, none of the mutations displayed appreciable alterations in the folding in solution or in the ability to destabilize vesicles to cause leakage of preloaded fluorescent markers. In addition, we have found that a triple mutation of these residues into glutamine or arginine prevents the effective translocation of the N-terminus. Thus, we suggest that C-terminal histidine residues play an important role in the formation of the final and functional inserted state fold of T-domain.

This work was supported by NIH grants GM-069783 (A.S.L), GM-29210 (A.F.) andAI-022021 (R.J.C.)

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RNA Editing of the IQ Domain in $Ca_V1.3$ Channels Modulates their Ca^{2+}-Dependent Inactivation

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Adenosine-to-inosine RNA editing generates molecular diversity and serves to regulate protein function via recoding of genomic information. Here, we report discovery of editing within CaV1.3 Ca2+ channels, well-known for low-voltage Ca2+-influx and neuronal pacemaking. Significantly, editing results in amino acid changes within the channel's IQ domain, a calmodulin-binding site mediating inhibitory Ca2+-feedback (CDI) on channels. The editing turns out to require RNA adenosine deaminase ADAR2 which recognizes a RNA duplex structure formed by the edited sites and intronic complementary sequence. The variable activity of ADAR2 potentially underlies a spatially diverse pattern of CaV1.3 editing seen across the brain. Edited CaV1.3 protein is detected both in brain tissue and within the surface membrane of primary neurons. Functionally, edited CaV1.3 channels exhibit strong reduction of CDI; in particular, neurons within the suprachiasmatic nucleus show diminished CDI, with higher frequencies of repetitive action-potential and calcium-spike activity, in wild-type versus ADAR2 knockout mice. Our study reveals a mechanism for fine-tuning CaV1.3 channel properties in CNS, which likely impacts a broad spectrum of neurobiological functions.

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The Interaction Between the Cell Membrane and Two Paddle Domains

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The interaction between the cell membrane and the putative "paddle" domains in two different voltage gated K^+-channels, the KvAP (from Aeropyrum pernix) and the HsapBK (human), has been investigated by circular dichroism, fluorescence and NMR spectroscopy. These peptides corresponds to the domains that move, within the membrane, as a response to a change in membrane potential, to open or close the channel pore. The secondary structures of both domains change after addition of 0.1 mM POPC or 0.1 mM POPC/POPG 7:3 large unilamellar vesicles, indicating interaction between the domains and the lipid vesicles. Furthermore, both domains, in particular the one derived from HsapBK, induce perturbations in the membrane. This is seen by fluorescence leakage experiments using vesicles with encapsulated calcein, indicating that the domains affect the barrier properties of the lipid bilayer. ^{2}H NMR spectra of magnetically aligned bicelles showed that the peptide derived from KvAP had no or little effect on bilayer order, while the peptide derived from HsapBK significantly affected the spectrum. This study demonstrates that the domains derived from two full-length voltage gated K^+-channels interact with the lipid bilayer. However, their modes and strength of interaction with the lipids may be different.

1240-Pos Board B10

Solute Specificity and Transport Kinetics of the Urea Channel from Helicobacter Pylori

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Helicobacter pylori's unique ability to colonize and survive in the acidic environment of the stomach is critically dependent on uptake of urea through the urea channel, HpUreI. Hence, HpUreI may represent a promising target for the development of specific drugs against this human pathogen. To obtain insight into the structure-function relationship of this channel, we developed conditions for the high-yield expression and purification of stable recombinant HpUreI. Urea efflux was measured in HpUreI-containing proteoliposomes using stopped-flow spectrometry to determine the kinetics and selectivity of the urea channel. The kinetic analyses revealed that urea conduction in HpUreI is pH-sensitive and saturable with a half-saturation concentration (or K(0.5)) of ~163 mM. The solute selectivity analysis indicated that HpUreI is highly selective for urea and hydroxyurea. Removing either amino group of urea molecules diminishes their permeability through HpUreI. Similar to urea conduction, diffusion of water through HpUreI is pH-dependent with low water permeability at neutral pH. Finally, a new model for HpUreI function is presented which accounts for these and other recently published data.

1241-Pos Board B11

Ion Channel Reconstitution

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Since the discovery of the possible solubilization of membrane proteins and their isolation from other membrane constituents (purification), different methods were developed to reconstitute ion channel proteins into artificial lipid bilayers. These membrane proteins were then fully functional when correctly oriented and inserted in a lipid bilayer. The reconstitution plays a central role in identifying and characterizing the mechanisms of action of membrane proteins. The activity of the membrane proteins is studied using electrophysiology using different methods e.g., the black lipid membrane. Therefore, the structure-function relationship can be investigated to better understand the biophysical properties of membrane proteins in vivo.

Using a glass surface containing a micrometer hole, the fusion of vesicles on the surface becomes an attractive method for electrophysiology and then to reconstitute membrane proteins into the lipid bilayer without denaturation. Then stable lipid bilayers are formed by bursting a GUV on the glass surface, forming a free-standing portion above the hole.

With this technique, we studied the biophysical and pharmacological properties of different ion channels, for example potassium channels (KcsA, Kv1.2), sodium channels (NachBac, NaVsp1) as well as other ligand-dependent (IP3 receptor, NMDA receptor), mechanosensitive channels (MscL, TRP channels) and non-specific channels (Cx43, VDAC). I will describe here our methods for incorporation of proteins into the bilayer and the recording of single ion channel current measurements by using a planar patch clamp platform.